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ANALYTICAL SFE APPLIED IN NUTRITIONAL LABELING ANALYSIS

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Summary

The recently mandated Nutritional Labeling and Education Act (NLEA) in the United States offers several opportunities for using supercritical fluid-based methods as an alternative technology to conventional analytical procedures employing organic solvents. Assays for total fat, fatty acids, fat soluble vitamins, cholesterol and fat replacers are ideal candidates for the application of supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). The research reported here is concerned with the development of a SFE-based procedure for total, saturated and monounsaturated fat. In these studies, SFE, using carbon dioxide, has been shown to be equivalent to classical procedures for the quantitation and speciation of the major components of fat. Extractions of homogenized ground beef samples with supercritical CO_2 have yielded equivalent results to those obtained with ethyl ether. The necessity of performing pre-extraction hydrolysis on the sample, followed by lipid speciation via gas chromatographic analysis of fatty acid methyl esters, was confirmed.

I. INTRODUCTION

Recent environmental legislation in the United States [1] has required a reduction in the use of number of common, yet carcinogenic or environmentally harmful solvents [2]. Coincidence of the above legislation with the Nutritional Labeling and Education Act [3] has created an opportunity for employing supercritical fluid techniques as replacement extraction or chromatographic solvents. NLEA assays, which are excellent candidates for the integration of supercritical methodology, include total fat (including its saturated and unsaturated constituents), cholesterol, fat soluble vitamins and synthetic fat replacers [4].

In this study, we have investigated the applicability of SFE as a replacement for traditional solvent extraction in the suggested NLEA protocol for total, saturated, and unsaturated fat. The NLEA method consists of hydrolytic treatment of the

sample prior to extraction to release "bound" lipids, followed by SFE or liquid extraction of the fat/oil. After isolation of the lipid extract, the fat sample is converted to the corresponding methyl esters of the constituent fatty acids of the extracted triglycerides, etc., via transesterification. Subsequent gas chromatographic analysis of these fatty acid methyl esters (FAMES) allows the quantitation of saturated and unsaturated fat after stoichiometric conversion of the fatty acids back to triglycerides [5]. This complicated method has been advocated as a replacement for traditional assays that are based on gravimetry. Results from gravimetric-based methods have been shown to be dependent on the choice of extraction solvent, due to the coextraction of non-lipid moieties.

To prove equivalence with the traditional method, we have undertaken a rigorous examination of the experimental factors which effect the accuracy and precision of the NLEA method using SFE. This has included the role of extraction pressure and temperature, the effect of cosolvent addition, quantitation of the lipid extract, and pre-extraction hydrolysis. The sample matrix of choice has been a highly homogenized ground beef which has also been analyzed with respect to its constituent fat content by the NLEA fat method. Sample uniformity was established by running extractions on subsamples of the homogenized meat sample by both SFE and conventional solvent extraction.

II. EXPERIMENTAL

SFE was performed on an apparatus designed and constructed at NCAUR for the extraction of large samples with supercritical carbon dioxide (SC-CO$_2$) [6]. Extraction conditions were 70MPa and 80°C, unless otherwise noted. Cosolvents were added by placing either a known amount of solvent directly into the extraction cell before SFE or dynamically adding the cosolvent with the aid of a Beckman 100A liquid pump (Beckman Instruments, Inc., Fullerton, CA). The meat samples were mixed with Hyromatrix [7] to aid in dispersing the sample prior to analysis. For certain extractions, the meat matrices were dehydrated to aid in the removal of the lipids.

Acid hydrolysis of the meat sample prior to SFE was accomplished using the method of Lembke and Engelhardt [8]. This procedure consisted of boiling a 2 gram meat sample in 80 mL of concentrated HCl and 100 mL of water. After boiling for 30 minutes, the mixture was gently filtered through a fluted 32 cm Whatman filter paper. After rinsing with 500 mL of distilled water, the paper was placed in a forced air oven and dried at 80°C. The dried filter paper was then cut into smaller pieces and placed into a tubular extraction cell [6] before SFE.

After extraction, the lipid extract is transferred to a 3 dram vial using 2 mL of chloroform and then 2 mL of diethyl ether. At this point, 1 mL of a C$_{19}$ triglyceride solution [8] was added to the sample and the excess solvent removed by N$_2$ sparging at 40°C. The formation of methyl esters for GC analysis followed the procedure of House [8], as did the GC analysis, except for subtle changes in the temperature programmed run. One microliter injections of the n-hexane layer, resulting from

the methyl ester workup, were used for GC analysis on a HP 5890 Series II (Hewlett Packard Co., Wilmington, DE). Individual fatty acid reference standards and/or mixtures (NuChek Prep, Elysian, MN) were purchased for calibrating the flame ionization detector (FID) used for the above analyses.

III. RESULTS AND DISCUSSION

Previously, we have demonstrated the analytical SFE is very effective for removing fats from a variety of foodstuffs [9]. For example, SFE has been performed on a variety of snack foods ranging in fat content from 1.5 - 50 weight %. Similarly, fat has been extracted quantitatively from meats ranging in fat content from 1.8 - 88 weight %; in some cases having a moisture content of over 75 weight %. In general, we have found very good agreement between label content and % fat as determined by gravimetry on $SC\text{-}CO_2$ extracts. The addition of cosolvents to $SC\text{-}CO_2$ for the determination of fat in snack foods and products, using processed or refined oils/fats in cooking or compounding operations, produces a negligible increase in the total fat content. This is probably due to reduction of polar and extraneous lipid moieties in the fat/oil during their production process.

Earlier SFE studies on ground turkey samples using either neat $SC\text{-}CO_2$, or $SC\text{-}CO_2$ with a cosolvent added to the sample or $SC\text{-}CO_2$, coupled with dehydration of the sample matrix, produced weight % fat results that varied from 10.7 - 19.6. These erratic results show the non-specificity of the extraction conditions for lipid matter when using gravimetry to measure total fat. For example, performing SFEs with cosolvents usually results in a higher weight % of fat over that recorded with pure $SC\text{-}CO_2$. This trend is not due to the removal of more lipid matter from the meat sample but results from the small but finite solubility of water and other coextractives that are soluble in the organic solvents, particularly in polar organic solvents. We have also noted that higher extraction pressures favor the solubilization of non-lipid coextractives and, hence, may lead to additional error in determining the true total fat content of the food product. Independent studies using the NLEA total fat method with liquid extraction have consistently shown the lowest weight percent fat for the above meat sample, of all the extraction methods. This is due to the speciation provided by the FAME analysis.

To properly integrate SFE into the NLEA total fat protocol, we have undertaken very precise extraction experiments under controlled conditions and performed GC/FAME analysis on the resultant extracts. NLEA total fat analysis was also performed on the same samples by Medallion Laboratories (Minneapolis, MN) using conventional liquid extraction. In order to eliminate any ambiguity in the sample matrix, extremely homogeneous meat samples (ground beef) were prepared in collaboration with the Department of Meat Science at the University of Illinois (Champaign, IL). The samples were prepared from beef trimmings by grinding them through a 13 mm plate, followed by mixing in a ribbon mixer, re-grinding through a 3 mm plate, with final homogenization in a bowl cutter.

Separate 125 gram packets of the above ground beef were taken for extraction and

analysis of fat content. One of the packets was analyzed individually while the other was divided into quarters for subsample analysis. Acid hydrolysis was performed on the beef samples, followed by SFE at NCAUR or ethyl ether extraction at Medallion Laboratories. Derivatization of these extracts via transesterification to form the fatty acid methyl esters gave the following results, as shown in Table 1 below. Column 2 lists the FAME results for the analysis of the single packet of meat. The individual fatty acid analysis for subsamples #1-4 are listed in columns 3-6. The excellent agreement in the fatty acid distribution of the single sample, as well as the subsamples, indicates that the SFE is reproducible, and that the overall sample is very homogeneous. This can be verified by comparing the average of the subsample analysis (column 7) with the result in column 2.

methyl ester	NCAUR %	NCAUR #1 %	NCAUR #2 %	NCAUR #3 %	NCAUR #4 %	NCAUR AVE %
8:0						
10:0	0.05	0.05	0.05	0.05	0.05	0.05
12:0	0.08	0.06	0.06	0.06	0.06	0.06
13:0						
14:0	2.49	2.54	2.53	2.54	2.53	2.53
14:1	0.69	0.72	0.72	0.72	0.72	0.72
15:0	0.51	0.51	0.51	0.51	0.51	0.51
15:1						
16:0	22.45	22.43	22.46	22.46	22.43	22.45
16:1	3.64	3.76	3.72	3.69	3.71	3.72
17:0	1.54	1.53	1.53	1.53	1.53	1.53
17:1	1.22	1.27	1.27	1.25	1.26	1.26
18:0	15.07	14.78	14.80	14.85	14.81	14.81
18:1c	44.00	43.70	43.99	44.13	44.27	44.02
18:1t	2.51	2.53	2.51	2.50	2.52	2.52
18:2	3.77	3.94	3.83	3.87	3.82	3.86
18:3	0.22	0.20	0.21	0.22	0.22	0.22
20:0	0.23	0.22	0.21	0.21	0.21	0.21
20:1	0.76	0.79	0.73	0.78	0.75	0.76
20:2	0.08	0.07	0.08	0.08	0.11	0.08
20:3	0.21	0.45	0.40	0.24	0.24	0.33
20:4						
22:0	0.18	0.18	0.17	0.15	0.16	0.17
22:1						
24:0	0.05	0.08	0.10	0.09	0.08	0.09
Total	100	100	100	100	100	

Table 1: Normalized FAME Analysis of Supercritical Fluid-Extracted Ground Beef Samples.

The fat results from our laboratory and Medallion Laboratories are compared in Table 2.

Type of Fat	Weight % Fat in Subsample				
	f1	f2	f3	f4	Avg. (RSD)
Medallion Labs					
Total	14.7	14.9	14.4	14.5	14.6 (1.4)
Saturated	6.0	6.1	5.9	5.9	6.0 (1.6)
Monounsaturated	6.5	6.6	6.4	6.4	6.5 (1.5)
SFE - NCAUR					
Total	14.3	15.1	15.4	14.9	14.9 (3.3)
Saturated	6.2	6.4	6.6	6.3	6.4 (3.1)
Monounsaturated	7.3	7.6	7.8	7.6	7.6 (2.6)

Table 2: Analysis of Subsamples of Ground Beef for Total, Saturated and Monounsaturated Fat Content

The agreement between the subsample analyses for those submitted to Medallion Labs and determined at NCAUR is excellent with average RSDs of 1.4 and 3.3%, respectively, for total fat. This precision, along with the precision associated with the saturated and unsaturated fat moieties, indicate a high degree of sample homogenization. A comparison of the data from the two laboratories for the same subsamples, as well as their respective averages, indicate that SFE can probably be substituted for the conventional liquid solvent extraction procedure in the NLEA method. The discrepancy between the values for the monounsaturated fat is probably due to chromatographic resolution in the C_{18} region, and hence quantitation problems in the GC/FAME analysis. It should be noted that an independent analysis of other beef packets gave 14.5 wt.% (Medallion) and 14.9 wt.% (NCAUR), in excellent agreement with the above subsample analyses.

It is interesting to compare the fat results from the NLEA method with those determined by gravimetric analysis of the collected fat. These results are shown in Table 3 below.

Technique	Sample f			
	f1	f2	f3	f4
Gravimetry	14.5*	15.6	15.9	15.5
NLEA Analysis	14.3	15.1	15.4	14.9
*All results in weight %				

Table 3: Comparison of Gravimetric Assay with NLEA Total Fat Method on Four Ground Beef Subsamples.

Here it can be seen that the gravimetric results tend, on the average, to be 0.5-0.6 wt.% higher than those computed from the NLEA analysis. We have consistently found this trend for most of the meat samples we have extracted to date.

IV. CONCLUSIONS

The above results show that SFE using SC-CO$_2$ can be substituted for conventional liquid solvents in the new NLEA method for total, saturated and unsaturated fat. Recent experiments designed to transfer the above method onto commercial SFE instruments (the HP 7680T extractor to date) appear to give equivalent results to those presented above. Additional research is being conducted with a new internal standard to improve the GC/FAME analysis and an alternative hydrolysis procedure to that presented above.

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DISCLAIMER

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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